TRANSPORT OF UREA BYERYTHROCYTES DURING HAEMODIALYSIS

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Haemodialysis constitutes a dialysis of a two-phase system: erythrocytes and plasma. Both phases contain urea. The plasma urea is carried to the concentration gradient in the dialysing membrane by diffusion as well as by turbulent convection, the latter being caused by the corpuscular elements of the blood. Urea diffuses out of the erythrocytes as soon as a concentration difference is established between the two phases by elimination of urea from the plasma into the dialysate. Therefore urea has to pass the erythrocytic membrane. The urea transport from erythrocyte to plasma is given by the equation:

$$\frac{da_{ue}}{dt} = -P_e \cdot S_e \cdot (c_{ue} - c_{up})$$  \hspace{1cm} (1),

where $da_{ue}$ is the amount of urea leaving the erythrocytes in the time $dt$, $P_e$ is the urea permeability of the erythrocytic membrane, $S_e$ is the surface of the erythrocyte, $c_{ue}$ and $c_{up}$ are the urea concentrations in the erythrocyte and plasma at the time $dt$.

During the passage of the dialyser a urea concentration gradient between erythrocytes and plasma is established, when urea diffuses into the dialysate and thereby $c_{up}$ diminishes. This concentration gradient is a function of the urea permeability of the erythrocytic membrane and a function of the elimination rate from plasma to dialysate. Direct measurement of the urea concentrations in erythrocytes and plasma leaving the dialyser would indicate which amounts of urea are transported from erythrocytes and plasma to dialysate. But the erythrocytes leave the dialyser in disequilibrium with respect to the urea concentration in plasma. They will transport urea into the plasma following the above equation until equilibrium is reached. This process is so fast that a suitable separation of the erythrocytes is not possible. However, the urea retention of erythrocytes during passage of the dialyser may be estimated indirectly. The degree of retention will diminish the extraction of urea by the dialyser in respect to whole blood. Urea analysis of the whole blood is not affected by urea diffusion between the two phases.

METHODS

In all experiments the Chron-A-Coil (Travenol) was used. The dialyses were performed in recirculated single pass as previously described (Kopp and Grossmann, 1966). The blood flow was determined by calibration of the revolution of the roller-pump. The mean hydrostatic pressure gradient between blood and dialysate was 300 mm Hg. Most data were gathered during dialyses in patients. Some data were gathered with blood of high haematocrits in vitro. The temperature of the dialysate was 36.5°C. Urea was determined enzymatically and the haematocrit by centrifugation.
TRANSPORT OF UREA BY ERYTHROCYTES DURING HAEMODIALYSIS

Fig. 1. The urea dialysances of whole blood in the Travenol Chron-A-Coil are plotted against the blood flow. The parameter for the different curves is the haematocrit indicated at each curve. The dotted line shows the dialysance for well-stirred plasma, gained by extrapolation of the increments in clearance from the measured curves to the haematocrit zero. The points are mean values from 4-6 single measurements. The urea concentration gradient from blood entering the coil to dialysate is in the range of 120-160 mg%. Higher gradients result in the same mean clearances, lower gradients, however, result in lower clearances: See Fig. 2.

RESULTS

The urea dialysance of whole blood as a function of blood flow and haematocrit is shown in Figure 1. Each point represents the mean value of 4-6 single measurements. The urea concentration difference between inflowing blood and dialysate was about 140 mg%. The urea dialysance increases with decreasing haematocrits. The urea dialysance for well-stirred plasma is obtainable by extrapolation at various blood flows. Of course, the urea dialysance of plasma estimated experimentally would be somewhat lower, the reason being that urea transport by convection is zero. The influence of convection is constant in the range of haematocrits examined.

In Figure 2 the same measurements of urea dialysances as in Figure 1 are shown, the difference being that the urea concentration gradient of inflowing blood to dialysate is only about 50 mg%. The urea dialysances of whole blood become thereby lower than with the high concentration gradient. The differences between extrapolated maximum dialysances at haematocrit = 0 and the measured dialysances are also present at lower blood flows. The urea transport ratio is given by the formula:

\[
\frac{\text{Urea transported by one erythrocyte-volume}}{\text{Urea transported by one plasma-volume}} = 1 - \frac{1}{\text{hct}} \left( 1 - \frac{D}{D_0} \right)
\]

where hct stands for haematocrit (as a fraction)

\[D\] measured urea blood dialysance
\[D_0\] extrapolated urea blood dialysance for haematocrit = 0 at the same blood flow.

This quotient is plotted in Figure 3 for haematocrit 0.20 at various blood flows for the two concentration gradients shown above. It is obvious that increasing blood flows diminish
**Fig. 2.** The dialysances for urea of whole blood are shown as a function of blood flow as in Fig. 1, the essential difference being the urea concentration gradient from blood entering the coil to dialysate. This gradient is in the range of 40-60 mg%.

**Fig. 3.** The ratio urea transported by unit-volume erythrocytes/urea transported by unit-volume plasma is plotted against the blood flow for the two urea concentration gradients from blood entering the coil to dialysate shown in Figs. 1 and 2. The ratios are calculated from formula (2) in the text. As there are two curves instead of one expected from formula (1), the urea permeability of the erythrocytic membrane is not a constant, but a function of the urea concentration gradient mentioned above. Higher gradients yield higher mean permeabilities and vice versa.
the ratio of urea transported by erythrocyte-to-plasma-volume: The higher the blood flow the shorter is the time erythrocytic urea is allowed to dialyse. However, following equation (1) the same function for any blood-to-dialysate concentration difference at constant haematocrit should result. Experimentally the assumption that the urea permeability of the erythrocytic membrane is a constant, is not valid. The urea permeability of the erythrocytic membrane is a function of the urea concentration gradient at this membrane. Low concentration gradients give lower permeabilities, high urea concentration gradients correspond to higher permeabilities. As demonstrated (Grossmann and Kopp, 1966), plasma urea decreases stepwise along the dialysing coil, corresponding to stepwise urea diffusion inhibition and facilitation in the erythrocytic membrane. The different permeabilities result in a stepwise binding capacity for urea at the n-alkyl chain in the biological membrane. The kinetics of repeated permeability variations may be approximated by a mean value for the erythrocytic permeability of urea. This is the case for high concentration differences. Low concentration differences in the range from 20 to 50 mg%, urea yield a permeability of the biological membrane for urea, which must be lower.

CONCLUSION

The transport ratio of urea by erythrocytes, compared with the plasma urea transport (volume to volume), is a function of haematocrit and blood flow rate. The erythrocytes retain urea during haemodialysis in the coil. The amount of retained urea increases with increasing haematocrit and decreasing time over which the erythrocytes are present in the coil. The urea permeability of the erythrocytic membrane is a function of the urea concentration gradient. In the usual haemodialysis techniques the amounts of urea transported per unit volume by erythrocytes are therefore only a fraction of the amounts of urea transported by the plasma.

REFERENCES


DISCUSSION

The Chairman (De Wardener, London): Dr. Rubini: I am not quite sure how much protein you want us to give.

Rubini (Los Angeles): We started off restricting protein: the patients do not like it. As we dialyse more, we give more and more protein. Our patients are on ad lib diets: I think they feel better for it and I see no deleterious effect.

Kerr (Newcastle): I should like to make a few comments on Dr. Rubini's paper. Firstly, the non-linearity in the rise of creatinine in urate seems to be largely confined to the first day, though there may be a little curve later on. I wonder whether this is not just a prolonged rebound for creatinine and urate after dialysis. Have you studied the early post-dialysis phase?

Secondly, some of the curve in the urea nitrogen rise must be due to the extrarenal losses that Dr. Deane and others have talked about and, in your neomycin-treated patients, I wonder whether you have studied stool urea content? I have been surprised at how high the stool urea is in the patients on neomycin, sometimes exceeding the blood urea level.

Thirdly, your study on the effect of doubling protein intake; was this a balance study in hospital or an out-patient exhortation to ‘double your protein intake’? I just make the observation that, in 24 of our patients who maintained they were eating 40 grammes of protein, a dietary survey showed an average intake of 58 g a day.

Rubini (Los Angeles): The linearity or the lack of linearity holds for uric acid and creatinine as well as urea. It is quite striking and, indeed, if you use logarithmic plots it almost looks as if it were asymptotic. Perhaps the slide did not show this characteristically. It is not a phenomenon of the first day, but the asymptotic features continue throughout the dialysis interval. As far as the protein intake is concerned, we have also had the same problem of estimating intake from what the patient is told to take and what he really is taking. However, when you put these people on an ad lib diet they very substantially increase their intake. We have tried a few balance studies, but our patients are working: it is very difficult to pull them out of a difficult sociological adjustment to get this kind of study. I am convinced, however, that they have very substantially augmented their intake. The problem is not whether it has actually doubled, but it is very much greater.

As far as the stool data is concerned we are worried about the methodological problems involved—whether you are really measuring urea even with urease methods under these circumstances. Sometime ago we studied sprue stools for urea content and it appears that there is substantial urea in stools.

The Chairman: Thank you Dr. Rubini. Is anybody going to stand up for controlled protein diet? I am surprised.

Rubini (Los Angeles): They would have two years ago.

The Chairman: After all we have had some very definite statements during the last 24 hours by people who have felt that the blood urea, at least at the beginning, should be not much more than 150, and you seem to be happy with blood ureas which are considerably greater. You say your patients are very well. We get others who say that if the blood urea is over 150 the patients are not so well. I am surprised at this discrepancy. Anyhow, you are in line with Dr. Comty.

254
DISCUSSION

KAYE (Montreal): Surely it is the relationship between the protein intake and the frequency of dialysis that matters? If you are going to dialyse often and for a long period of time you can get away with virtually any protein intake. We have a young girl who is on an unrestricted diet taking at least 70 or 80 g of protein a day. She is only 14, but she is getting dialysed every day. I think once your blood urea nitrogens are over 100 mg of pre-dialysis, you are liable to get difficulties in the way of post-dialysis disequilibria. I should like to ask Dr. Rubini if he has considered whether there may be an adaptive response through the skin. Does he have any measurements on urea, creatinine, etc., lost by sweating? Los Angeles is, I believe, a rather warm area.

RUBINI (Los Angeles): We have gone further than that and tried to use the sauna bath and have found some interesting data. Urea does go through the skin, uric acid and creatinine do not. The amount lost can be significant if you induce substantial sweating. Los Angeles is not that warm and people adapt their sweating mechanism. I would doubt if there are very substantial urea losses through the skin. Certainly, sensible perspiration—and I think one would need to visualise perspiration—is not very great in these patients.

RAE (Seattle): I should like to confirm again what we said yesterday about the amount of protein we give our patients to eat on chronic dialysis. Most of our patients have gone up to 80 g. Perhaps it would be more accurate to have said that they are on a free diet. Some of them will presumably be taking more than this and some will be taking less. The key to the situation, as Dr. Kaye has pointed out, is that you dialyse the patient frequently so that there is not too great a shift in each dialysis of urea and other substances. I have recently had the opportunity of seeing a number of patients who were under-dialysed in another area and who were also on a fairly free protein diet. They were being dialysed twice a week for a short time and four of them manifested quite definite symptoms which I presume were due to disequilibrium, terminating their dialyses on each dialysis after four or five hours because of headaches and vomiting.

KOCARI (Istanbul): I ask this question to Dr. Scholz and Dr. Deane together, I should like to know if the production of urea takes place in the kidney too, because I believe this is the case. If this is so, what percentage is produced in the kidney?

DEANE (New York): We have not had an opportunity to study the bilaterally nephrectomised humans pre and post nephrectomy, so I am unable to answer how much of urea production might be accounted for by the kidney. I would suspect it would be small.

The CHAIRMAN: Dr. Deane, have you any explanation why, if you make a patient better on a Giovannetti diet, his ability to use urea is better whereas with dialysis it is worse?

DEANE (New York): I am not sure we can explain that at the moment, but if you view the equilibrium involved, when you haemodialyse the patient, you are dropping one end of the equilibrium and if you alter the protein intake drastically you are influencing the other end of the equilibrium.

I should like to add that I think that Dr. Rubini's paper and other information presented here suggest that we should seriously consider the concept that the patient on chronic haemodialysis is not simply the individual with stable chronic renal failure, whose function has worsened sufficiently to require this kind of permanent assistance. Possibly this patient then becomes an individual who is metabolically quite different from the patient with stable chronic renal failure. Obviously this is a hypothesis that has to be tested.

KOPP (Frankfurt): I should like to make a few comments on the papers dealing with the pool
DISCUSSION

and distribution of urea in the body. I would quite agree with the fact that the turnover rate is not markedly increased except in cases of frank hypercatabolism. On the other hand, I wonder about the values of distributions they found. The distribution volumes may be easily calculated by dividing the total quantity of urea which is extracted during one dialysis by the concentration difference during any given time and this method shows a larger volume of distribution.

I think a possible explanation may be given also for the lack of correspondence between the increase in blood urea after dialysis and the estimated urea production rate. This is that in fact urea is not freely diffusible and not equally distributed, as we demonstrated in last year's proceedings. We think that urea can be bound up inside biological membranes, by the molecular layer of phospholipids as an addition compound and therefore the increase of urea by production is not immediately followed by an increase in the serum levels since the urea flux into the tissues remains on a plateau as long as the binding capacity of the membranes for a certain concentration is not saturated.